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*Full Length Research Paper*

# Developmental biology and field performance of *Platygaster diplosisae* Risbec (Hymn: Platygasteridae) an egg-larval parasitoid of african rice gall midge *Orseolia oryzivora* Harris and Gagné (Diptera: Cecidoymiidae)

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**The developmental biology and field performance of *Platygaster diplosisae* an endoparasitoid of African rice gall midge (AfRGM) *Orseolia oryzivora* were investigated. Experiments were conducted in the screen house, laboratory and fields during 2006/2007 farming seasons. The pre-oviposition period averaged 12.1 h. The mean number of eggs laid per female was 95.2 and mean oviposition period was 3.5 days. The average incubation period was 13.0 days with a hatchability of 67.3%. There were three larval instars with developmental duration averaging 2.2, 0.5 and 2.5 days for the 1st, 2nd and 3rd larval respectively. The prepupa and pupa developmental period lasted for 2.4 and 6.7 days respectively. The longevity of males and females were 3.0 and 4.1 days respectively without food, which varied when food was given. The parasitoid has total developmental period of 27.3 days. The results showed that *P. diplosisae* has an impact as a bio-control agent of AfRGM, but the percentage parasitism of the host was low at the beginning of the season and increased significantly later in the season with a peak in October of each season. It was recommended that mass rearing or conservation of the parasitoid be adopted to improve its efficiency in control of AfRGM.**

**Key words:** Biology, *Platygaster diplosisae*, bio-control, field impact, *Orseolia oryzivora.*

## INTRODUCTION

Rice is now the main staple food for about 35 million people or 20% of the African population, and consumption is increasing faster than that of any other food crop in many countries in Africa (Kormawa et al., 2004; Mohapatra, 2006; WARDA, 2008). Unfortunately, rice production and yield has been dwindling and has failed to meet the domestic demands in many African countries.

The production-consumption gap in Africa has been attributed to insect pest infestations and inferior quality of domestic rice (Ogah et al., 2009). Insect pests are some of the major constraints in achieving the yield potentials of many varieties of rice. Of all the insect pests of rice, African rice gall midge (AfRGM), *Orseolia oryzivora* Harris and Gagné (Diptera: Cecidomyiidae), is the most serious insect pest of lowland and irrigated rice in the recent time (Ogah et al., 2005; Nwilene et al., 2006; Omoloye and Vidal, 2007). Losses caused by this pest have reached 80% and total crop failure is common in endemic areas in Africa (Heinrichs and Barrion, 2004).

Insect pest management in Africa characterised with the use of insecticides and some few other cultural measures have not given the desired results in the management of

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most insects for example, AfRGM. Development and implementation of appropriate AfRGM management approach is therefore an absolute necessity.

Consequently, several studies have been undertaken to identify appropriate control measures for AfRGM. Biological control seems to be the most promising option of controlling AfRGM. Diverse complexes of natural enemies of AfRGM have been identified in Nigeria and other West African countries (Ogah et al., 2009). Two most important natural enemies of AfRGM identified in the fields are the parasitoids. The two promising parasitoids of the AfRGM include *Platygaster diplosisae* Risbec (Hymenoptera: Platygastridae), a gregarious egg/larval endoparasitoid and *Aprostocetus procerae* Risbec (*Tetrastichus pachydiplosisae*) Risbec (Hymenoptera: Eulophidae), a solitary pupal ectoparasitoid. Field surveys of natural enemies of AfRGM have reported the activities of *P. diplosisae* as an important bio-control agent of AfRGM (Umeh and Joshi, 1993; Ogah et al., 2009). Parasitism caused by these two parasitoids can reach as high as 77% of the immature populations of the pest. These potentials could be trapped and used as bio-control agents of AfRGM (Bâ, 2003; Ogah et al., 2009).

In the development of biological control techniques, understanding the biology of the organisms is a yardstick for selecting suitable natural enemies to be used for efficiency. The biology of AfRGM has been studied by many workers, however little information has been published on the developmental biology of *P. diplosisae* the most promising bio-control agent of AfRGM in Africa. The present study was conducted with the objectives of providing more information on the developmental biology of *P. diplosisae,* and its field performance as a bio-control agent of AfRGM.

## MATERIALS AND METHODS

### Experimental sites/materials

The experiments were conducted at Africa Rice (WARDA) in IITA Ibadan Nigeria, using their screen houses and laboratories, under ambient conditions of 23 to 33°C and 37 to 75% relative humidity. The field experiments to determine the impact of the parasitoid on bio-control of AfRGM was conducted at two endemic areas (Ogidiga, Southeast and Edozhigi, Northcentral) in Nigeria.

In order to have adequate numbers of insects required for the experiments readily as at when needed, two stock cultures were prepared under ambient conditions. These were the:

1) *O. oryzivora* culture, and
2) *P. diplosisae* culture

### *O. oryzivora* stock culture

A susceptible rice variety, ITA 306 was used for the production of stock culture. The seedlings were raised in seed boxes and were placed in an oviposition cage in the WARDA screen house at international institute of tropical agriculture (IITA) Ibadan. Galled tillers were obtained at the experimental fields at Ogidiga, Ebonyi State and were transplanted into the seed boxes at Ibadan. These were kept in an oviposition cage and later transferred into the emergence cage in the screen house for emergence. Twenty *O. oryzivora* females that have been allowed to mate for 12 h were carefully selected using a glass vial (2.5 × 8.0 cm diameter) and were introduced for oviposition on the seedlings in the seed boxes at 14 days after seeding. They continued to oviposit in the seedling until they died. Thereafter, the seedlings were sprayed with water manually using a Hills Master$^{R}$ sprayer at two hours interval continuously during the day between 6.00 and 18.00 h GMT daily for three days to facilitate entry of the *O. oryzivora* larvae into the rice culms. The seedlings were then transferred and placed in the screen house till the galls start to appear, after which they were transferred back into the emergence cages. Emerging adults were carefully collected with the aid of glass vials (2.5 × 18.0 cm) between 8.00 and 9.00 h GMT daily. The emerged adults were used to infest fresh batches of the susceptible seedlings to maintain a continuous clean and healthy culture.

### *P. diplosisae* stock culture

The culture of *P. diplosisae* was prepared in a screen house using seed boxes as described earlier. Parasitized galls collected from the experimental fields were transferred to WARDA screen house at IITA Ibadan. The parasitized plants were transplanted into seed boxes in the emergence cage for emergence. Upon emergence, twenty newly mated females of *P. diplosisae* were selected using glass vial (2.5 × 18.0 cm) and introduced into the *O. oryzivora* infested seedlings in a screen house in mass culture cage for parasitization. One of the stock culture cages of *O. oryzivora* containing newly infested seedlings of ITA 306 with *O. oryzivora* eggs was used at a time.

The mass culture cage was watered at every 2 h intervals to facilitate the hatching and penetration of the parasitized larvae for 3 days. The culture was maintained and upon emergence of the parasitoids, the adults were collected. The collected adults were used for further mass rearing.

### Developmental biology of *P. diplosisae*

To study the developmental processes of *P. diplosisae*, caged rice seedlings (ITA 306) infested with 40 one-day-old *O. oryzivora* eggs were used. Ten newly emerged females that were allowed to mate were collected from the stock culture cage; each was introduced into the cage with the *O. oryzivora* infested seedling for 24 h to determine the maximum number of *O. oryzivora* that a single parasitoid could parasitize. Each cage represents a replicate. After 24 h of exposure, the parasitoids were discarded. The experiment was carried out under ambient temperature and RH with natural light providing, a photoperiod of approximately 15 L: 9 D. Thereafter, the hosts were examined for parasitism (parasitoid puncture marks and colour change) under microscope. Observed parasitized hosts were all transferred into caged rice seedlings in the screen house using camel hairbrush. As the parasitized *O. oryzivora* larvae develop inside rice galls, each day ten rice seedlings each containing parasitized midge larva were dissected using SH – ZT binocular dissecting microscope. As the developing *O. oryzivora* larva was being extracted from the galls, they were killed in 60% alcohol and dissected immediately in 0.9% saline solution to study the developmental stages of the *P. diplosisae* using microscope. Parasitoids larvae in prepupal and pupal stages were recovered from the cocoons in their hosts for study. The body forms and gross features of immature stages of the parasitoids were recorded. The remaining observed parasitized hosts in the caged seedlings were kept in the screen house until the emergence

**Table 1.** Sizes (mm) of the immature stages of *P. Dilposisae.*

| Stage | Mean±SE (mm) | |
|---|---|---|
| | Length | Width |
| Egg | 0.03±0.01 | 0.01±0.00 |
| Larva: 1st | 0.43±0.04 | 0.23±0.01 |
| 2nd | 0.64±0.06 | 0.29±0.02 |
| 3rd | 0.84±0.07 | 0.37±0.03 |
| Prepupa | 1.01±0.01 | 0.44±0.02 |
| Pupa | 1.02±0.03 | 0.32±0.01 |

**Table 2.** Mean duration (days) of each developing stage of *P. diplosisae.*

| Stage | Mean duration (days) | |
|---|---|---|
| | Range | Mean ±SE |
| Egg | 12-14 | 13.1±1.1 |
| Larva: 1st | 1-3 | 2.2±0.4 |
| 2nd | 1-2 | 0.5±0.3 |
| 3rd | 2-4 | 2.5± 0.2 |
| Prepupa | 2-4 | 2.4±0.3 |
| Pupa | 6-8 | 6.7±0.2 |

of adult *P. diplosisae* and developmental period computed. All eggs, larvae, pupa and emerged adults of *P. diposisae* were measured using an olympus SH – ZT stereo binocular microscope fitted with an ocular micrometer.

Differences in the duration of the development between male and females were analyzed using a simple t -test. Adult longevity was estimated based on the observation of 114 individuals (56 females and 58 males).

#### Effect of food on longevity of *P. diplosisae*

To study the effect of food on the longevity of adults of *P. diplosisae,* newly emerged adults were used. The newly emerged adults were kept without either honey or water, or given water alone, honey alone, or water plus honey. Each test was conducted in an oviposition cage with 10 males and 10 females replicated five times. The mortality of each sex was recorded daily till death.

#### Percentage parasitism of *O. oryzivora* by *P. diplosisae* in the field

To determine the performances of *P. diplosisaae* as a bio-control agent of *O. oryzivora* in the field, experiments were conducted at two locations (Ogidiga, southeast and Edozhigi, North central Nigeria) identified as AfRGM hot spots (endemic areas) during the 2006 and 2007 wet seasons.

The experiments were performed using farmers' rice fields. The area planted to lowland rice at each location averaged 0.5 ha and planting was scattered starting from May to August of each season in the two locations. The rice variety grown at Ogidiga was Cisadane – a popular gall midge variety released as FARO 51 in Nigeria. At Edozhigi, ITA 306 also known as FARO 37 was used. Both varieties are susceptible to the AfRGM. Insecticides were not applied on any of the fields sampled.

Sampling for AfRGM infestation was conducted at monthly intervals at both locations from June through November of 2006 and 2007. For each field sampled, 50 plants were randomly selected to assess the intensity of damage due to AfRGM (percent tiller infestation) and dissected for percent parasitism (parasitized larvae and pupae and parasitoid species present).

Statistical analysis An arcsin transformation was used on percent tiller infestation and percent parasitism data prior to analysis of variance (SAS, 2003). The mean separation was carried out by Turkey's studentized range test. Pearson correlation coefficient (r) was used to determine the relationship between percent tiller infestation and parasitism by *P. diplosisae*.

## RESULTS

### Developmental biology of *P. diplosisae*

The developmental biology of *P. diplosisae* consisted of the egg, three instar larval stages, a prepua, the pupa and the adult stage. Because of its cryptic, internal placement, the egg was difficult to observe shortly after oviposition. The development of its newly deposited eggs in the host eggs was clarified based on the morphological characteristics. Initially, the egg is smooth and translucent, bluntly rounded at one end and slightly tapered at the other. Based on 10 individuals, the mean length is 0.03 mm and width at the widest 0.01 mm (Table 1). The eggs are free within the host egg, but later attach to the nervous system or midgut of the host larva. The eggs do not hatch until the host reaches the third larval stage.

Shortly after growth commences, many paranuclear masses are formed and the developing *P. diplosisae* embryo becomes encysted within host tissues, absorbs sustaining nutrients from them. When the first instar larvae were completely formed, they broke away from surrounding trophamnion and initiated feeding. The egg stage lasted about 13 days (Table 2). *P. diplosisae* hibernate in the early embryonic stage in the first instar larvae of the host. As the *O. oryzivora* larva develops, the wasp egg hatches into larva. The wasp is polyembryonic and so an egg gives rise to so many larvae within a single host. The larva has three larval instars stages.

**Table 3.** Mean longevity (days) of adult *P. diplosisae* with different food.

| Food sources | Sex | N | Longevity (day) |
|---|---|---|---|
| Honey and water | Male | 20 | 4.7[a] |
| | Female | 20 | 3.9[a] |
| Honey only | Male | 15 | 3.5[b] |
| | Female | 15 | 4.3[b] |
| Water only | Male | 10 | 3.1[c] |
| | Female | 10 | 4.2[c] |
| Nothing | Male | 12 | 3.0[c] |
| | Female | 12 | 4.1[c] |

Means followed with same letter are not significantly different (Student–Newman–Keuls test).

When fully developed the larva was white and ovoid, devoid of setae, hymenopteriform and about 0.84 mm length and 0.37 mm wide. The larva prefers to attach itself either to the ganglion or stomach of the host larva. There were three thoracic and seven abdominal segments. As *P. diplosisae* begins to feed on the tissues of the *O. oryzivora* host, it becomes progressively sluggish; finally it loses all power of locomotion, and eventually dies. During the feeding period, the larva increases somewhat in size and develops fat bodies and moult severally. The mouth was a simple transverse orifice. After killing the host larvae by entirely consuming the body contents, the *P. diplosisae* larvae enter the prepupal stage within the oblong, yellow-brown integument of the host puparium and form ovoid cocoon. Before pupation, each larva formed an ovoid cocoon where it pupates.

Parasitized *O. oryzivora* larvae are filled with wasp cocoon and are much bigger than healthy (unparasitized) *O. oryzivora* larvae. This larva stage lasted for an average of 5.2 days followed by the prepupa stage. The prepupal stage is average of 2.4 days period between cessation of feeding and the transformation to the pupa. The mature prepupa is white in colour. Three body segments are discernible. The mean length of 10 individuals was 1.01±0.01 and width 0.44±0.02. Initially the *P. diplosisae* pupa is white; the eyes soon darken, and the entire body, with the exception of the integuments between the abdominal plates gradually assume the shiny black colouration of the adult. The pupa stage takes on average of 6.7 days. The mean length of 10 individuals observed was 1.02±0.03 and width 0.32±0.01.

Adult wasps emerge from their cocoons by cutting tiny individual emergence holes (0.4 mm in diameter) in the gall through where they escape. Forty to 65 parasitoids emerged from each parasitoid host and the parasitoids were dominated by the same sex from the particular parasitoid host. After emergence, empty cocoons were seen inside the gall. The newly emerged adults remained in the host for about four additional days before escaping from the galls. The mean length of ten adult females was 1.6 mm and males 1.3 mm. The longevity of adult female was 4.1 days while male was 3.0 days. The complete life cycle that is, from egg to adult emergence averaged 27.3 days at ambient laboratory temperature and relative humidity. The female adult has shiny black colouration while the male was dark brown and smaller in size. An adult *P. diplosisae* has short antennae and 10-segmented with moderately long scale, and at least 4 antennal apical segments light brown. The posterior part of the thorax was densely setose laterally, whereas the forewings are without veins. Legs are pale yellowish brown and tibiae II and III with a-one long spur.

Based on the observation made using 10 adult females, *P. diplosisae* is a prosynovogenic species (that is a species for which the process of maturation of its eggs starts during the pre-immature stages and continues after the adult has emerged from its host). Egg counts were made from ovaries dissected from 10 *P. diplosisae* females. The eggs are so small and numerous that counts needed to be made by spreading the eggs in fluid on an ocular micrometer disk with a 10 × 10 mm square-grid recticle. The average number of egg counts in the ovaries per female was 648 but only 95.2 eggs were laid per female during her life time.

## Effect of food on longevity of *P. diplosisae*

Food availability greatly influenced adult longevity (Table 3). However, there was no significant difference (P=0.05) between longevity of male and female parasitoids under any of the conditions provided. Generally, females lived longer than males in all the experimental conditions.

## Field performance of *P. diplosisae* as a bio-control of AfRGM

The impact of *P. diplosisae* as a bio-control was based on the percentage AfRGM infestations in the field parasitized. The infestations varied significantly among the sampled sites and years. Generally AfRGM infestation was less frequent during the initial stages of the survey at both sites but increased with time. Edozhigi in the moist savannah zone had highest percentage infestation recorded in October of each season (Table 4). The trend was the same at Ogidiga savannah/forest transition zone where infestation increased rapidly from August to October and decreased afterwards.

During the field experiments, two species of hymenopterous parasitoids were found as natural enemies of rice gall midge; *P. diplosisae* and *Aprotocetus*

**Table 4.** Mean (±SD) percentage *O. oryzivora* infestation and parasitism by *P. diplosisae* at Edozhigi/ Ogidiga during 2006/2007 seasons.

| Months | 2006 | | | | 2007 | | | |
|---|---|---|---|---|---|---|---|---|
| | Edozhigi | | Ogidiga | | Edozhig | | Ogidiga | |
| | % Infestation | % Parasitism | % Infestation | % Parasitism | % Infestation | % Parasitism | % Infestation | % Parasitism |
| June | 0.00±0.00 | 0.00±0.00 | 1.57±1.81 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| July | 4.08±2.52 | 3.63±3.41 | 6.55±1.30 | 1.15±2.57 | 3.68±2.31 | 2.39±2.46 | 5.11±1.92 | 0.00±0.00 |
| August | 9.28±2.86 | 10.08±1.76 | 12.44±5.97 | 16.44±7.34 | 7.64±1.23 | 6.13±2.48 | 10.76±4.19 | 3.23±4.39 |
| September | 27.47±4.63 | 27.50±8.05 | 31.84±1.89 | 28.13±2.65 | 22.57±7.41 | 21.31±7.64 | 29.84±2.80 | 13.81± 5.67 |
| October | 41.46±6.03 | 53.03±8.95 | 41.06±3.87 | 46.70±9.31 | 38.46±3.86 | 45.50±8.85 | 38.52±2.98 | 38.30±12.40 |
| November | 34.29±8.82 | 33.72±7.46 | 35.34±7.73 | 34.51±5.25 | 32.76±7.27 | 28.92±6.68 | 33.90±7.02 | 29.06±9.38 |

*procerae*. Parasitisms by the two are easily differentiated by the gall surface structure. Results showed that *P. diplosisae* was the most abundant parasitoid parasitizing AfRGM in the two locations throughout the experiments and was the only result presented in this report. The percentage parasitism by *P. diplosisae* followed the same trend at both sites. At Ogidiga the same parasitoid abundance trend was recorded, however percentage parasitism was much lower and delayed till September, then increased very abruptly after that reflecting the late but very rapid build up of AfRGM observed there. In October its population got at its peak to coincide with the peak of AfRGM infestation (Table 4). Beginning from November, the populations of both parasitoid and AfRGM were observed to decline slowly. At Edozhigi percentage parasitism by *P diplosisae* was delayed by two months after which parasitism increased from ending of August to October across years following increase in gall density with the highest parasitism at October for 2006/2007 farming seasons respectively (Table 4). There was a positive correlation between AfRGM infestation and % parasitism by *P. diplosisae* (r = 0.89, P< 0.001).

## DISCUSSION

*P. diplosisae* is a gregarious endoparasitoid and reproduction was facultative parthenogenetic. The number of ovarian mature eggs was 648, however the average number of eggs laid per female was 95.2. The complete life cycle averaged 27.3 days. Newly emerged adults remained in the host for about four additional days before escaping from the gall. Similar observation has being recorded for *P. oryzae* that parasitize *O. oryzae* in Asia by Hidaka et al. (1988). The parasitoid took about 5 days longer than the development of the host. This constitutes a serious weakness in the *O. oryzivora* and *P. diplosisae* population asynchrony which enables *O. oryzivora* population build up beyond levels which *P. diplosisae* can effectively control especially early in the season unless other parts of its life table attributes compensated for it.

The female biased sex ratio observed in *P. diplosisae* may be attributed to the haplo-diploid genetic system of sex determination in hymenoptera (unfertilized eggs give males and fertilized eggs give females). Females control the sex of their progeny by selectively releasing sperm from the spermatheca. Again the ratio may also be attributed to sex ratio distortion factor like son killer. This is a bacterium which I f a female parasitoid is carrying it; it kills all the male progeny at larva stage.

The results showed that *P. diplosisae* is an important agent for the control of AfRGM. The role of *P. diplosisae* as a natural regulator of the *O. oryzivora* have been reported by several authors (Bâ, 2003; Williams et al., 1999).

The results and those of earlier studies (Ukwungwu and Misari, 1997) suggest that *P. diplosisae* is an indigenous parasitoid with the potentials as natural bio-control agents of *O. oryzivora*. Umeh and Joshi (1993) has reported that the decline in gall density observed in the field as parasitism increases to give inverted sigmoid shaped curve could be attributed to the roles of the parasitoid. However, such high level of parasitism was recorded late in the season when most of the crop had been attacked suggesting a lack of synchrony of the parasitoid with that of the host in the field.

## Conclusion

In conclusion, the results of this study showed that *P. diplosisae* has great impact on the population of AfRGM in the field and could be used as its bio-control agent. It could therefore be recommended that based on the understanding of the lifecycle of *P. diplosisae,* a practical steps be taken towards getting local farmers to appreciate the importance infestation recorded in October of each season (Table 4). The trend was the same at Ogidiga savannah/forest transition zone where infestation increased rapidly from August to October and decreased afterwards.

During the field experiments, two species of hymenopterous parasitoids were found as natural enemies of rice gall midge; *P. diplosisae* and *Aprotocetus procerae*. Parasitisms by the two are easily differentiated by the gall surface structure. Results showed that *P. diplosisae* was the most abundant parasitoid parasitizing AfRGM in the two locations throughout the experiments and was the only result presented in this report. The percentage parasitism by *P. diplosisae* followed the same of the parasitoid in the farm by conserving it to make it more efficient in the management of AfRGM.

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